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Authors
Aramant, RB
Seiler, MJ
Ball, SL

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Successful Cotransplantation of Intact Sheets of Fetal Retina with Retinal Pigment Epithelium

Robert B. Aramant,1,2 Magdalene J. Seiler,1,2 and Sherry L. Ball1

PURPOSE. Many retinal diseases, such as macular degeneration, affect both retinal pigment epithelium (RPE) and photoreceptors. Therefore, retinal repair may require transplantation of both tissues together as a cograft.

METHODS. As recipients of retina-RPE cografts, 7- to 10-week-old albino Royal College of Surgeons rats that lose their photoreceptors because of a pigment epithelium defect were used. Freshly harvested intact sheets of RPE with neural retina from pigmented normal rat fetuses were gel embedded for protection and transplanted into the subretinal space.

RESULTS. After 6 to 7 weeks, with the support of the cografted RPE sheet, transplanted photoreceptors developed fully in organized parallel layers in the subretinal space. Immunohistochemistry for rhodopsin, rod α-transducin, and Santigen and peanut agglutinin labeling for cone interphotoreceptor matrix domains suggested that the photoreceptors in the graft were capable of normal function.

CONCLUSIONS. Freshly harvested intact sheets of fetal RPE and retina, transplanted together into the subretinal space, can develop a normal morphology. Such transplants have the potential to benefit retinal diseases with dysfunctional RPE and photoreceptors. (Invest Ophthal Vis Sci. 1999;40:1557-1564)
The animals were treated according to the regulations in the University Laboratory Animal Care and Use of Laboratory Animals. Donor tissue was obtained from pigmented embryonic day (E)18 to E20 Long-Evans rat fetuses prelabeled in utero by injecting a timed-thymidine analogue, on 2 to 3 gestational days before harvest. The donor tissue was then incubated in dispase (Collaborative Biomedical Products, Bedford, MA) for 10 minutes at 37°C to enable the RPE and retina to be dissected from the embryos. Donor eyes were then immersion fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 N Na-phosphate buffer (pH 7.2); eyecups were embedded in paraffin. The eyes of three rats were immersion fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 N Na-phosphate buffer (pH 7.2), and retinal pieces were embedded in Epon. Epon sections (0.75 μm) were stained with toluidine blue. Paraffin sections (8 μm) containing the transplant and the surrounding host retina were stained with hematoxylineosin, peanut agglutinin (PNA) lectin (1:1000; Vector, Burlingame, CA), and monoclonal antibodies for BrdU (Dako, Carpinteria, CA), rhodopsin, rod α-transducin, and Santigen (arrestin). The immunohistochemical procedures have been described previously.17,28

RESULTS
Transplantation Successes and Failures
In 4 of 11 animals (43 and 51 days after transplantation), the transplanted eyes contained cografts of fully laminated neural retina supported by the cotransplanted sheet of RPE (Figs. 2, 3, 4). In the remaining seven animals (data not shown), the RPE had separated from the neural retina during implantation, the neural retinal transplants had formed rosettes, and/or the transplanted RPE cells had dispersed and migrated into the retinal cografts. In three of these animals, transplants had been misplaced into the choroid and showed infiltration of macrophages.

Recipient Retina
At the earliest time of death (host age 3 months), the recipient retina had lost almost all rod photoreceptors (Figs. 1B, 1C, 1D). Although remnants of the outer segment debris stained for rhodopsin, no rhodopsin-stained photoreceptors could be seen (Fig. 1C). The sparse staining for the phototransduction protein Santigen in the recipient retina revealed only a few remaining cell bodies of photoreceptors, most likely cones (Fig. 1D).

Organization of Successful Cografts
In the four successful transplantations, a full lamination had developed in the neural retinal transplant with parallel retinal layers, including mature photoreceptors in contact with the cotransplanted RPE sheet (Figs. 2A, 2B, 2C). The donor tissue could be unequivocally identified by immunohistochemistry for BrdU. BrdU-labeled cells were seen in all retinal layers (Fig. 2D). In transplanted RPE cells, the BrdU-label was mostly obscured by the pigment and could only be seen in some less pigmented cells (Fig. 2E). Some ganglion or amacrine cells were misplaced in the inner plexiform layer of the transplant (Figs. 2A, 2B). The photoreceptor layer contained 5 to 10 rows of photoreceptor nuclei (Fig. 2C). Parallel inner and outer segments developed in transplant photoreceptors in contact with the cotransplanted RPE (enlargements shown in Figs. 2F, 3C, 4C, 4D). A sectioning artifact was seen in Figures 2A, 2D, 2E, 2F, 3A, 3B, 4A, 4B, 4C, and 4D (breakup of outer segments) which can also occur in sections from normal retina.

Interface between Transplant and Host Retina
The apposition of the transplants toward the host retina varied. In three of four transplants, partial absence of glial barriers and
interdigitation of transplant and host tissue could be seen (Fig. 2B). However, an apparent glial limiting membrane was seen separating part of the transplants from the host retinas (Figs. 2A, 2C). The fourth transplant was completely separated from the host retina by a glial limiting membrane. No remnants of host photoreceptor debris were seen in the transplant area. The neural retinal transplant and the overlying host retina contained some microglial cells, but no major immune response was seen (Fig. 2B). Some pyknotic ganglion cells were seen in the host retina overlying the transplant (Fig. 2B) but also in other areas.

**RPE Cells in Cografts**

The cotransplanted RPE sheets had been placed either onto the host Bruch’s membrane (Figs. 3A, 3C), or the transplanted RPE cells apparently had produced a second Bruch’s membrane on top of presumably remnant host RPE (Figs. 3B, 3D). These cells were suggested to be of host origin because of the intact sheet of pigmented donor RPE overlaying it. The remnant albino host cells had taken up some pigment granules from the donor cells (Fig. 3D). In either case, the transplanted RPE sheet maintained its characteristic monolayer. In one area of a transplant (not shown), the RPE sheet appeared to have folded on itself and formed a double layer. This did not interfere with the full development of the adjoining photoreceptors, although the outer segments were shorter in one case (Fig. 3D). One transplanted RPE sheet had a hole at the time of implantation. This hole appeared to have been sealed at the time of the animal’s death, presumably because of proliferation and/or migration of the transplanted RPE cells.

**Photoreceptor Markers in Transplants**

As a measure of photoreceptor organization and viability, transplanted photoreceptors were stained for the phototransduction proteins: rhodopsin (Fig. 2F), rod α-transducin (Figs. 4A, 4B), and S-antigen (Fig. 4C). Intense transducin immunoreactivity was seen in inner segments of fully developed photoreceptors organized in parallel layers and in contact with the transplanted RPE (Fig. 4A). The retinal edges of the transplants sometimes formed rosettes (Fig. 4A). In rosettes, photoreceptors had only weak transducin immunoreactivity (Fig. 4A). Cone interphotoreceptor matrix domains were labeled with PNA, suggesting a normal level of RPE-photoreceptor adhesion (Fig. 4D).

**DISCUSSION**

This study is the first to show that freshly harvested intact sheets of fetal RPE with neural retina can be transplanted to...
the subretinal space and survive, with development of an apparently normal cytoarchitecture. Previously, it has been shown that cогrafted cell aggregates of fetal rabbit neural retina with RPE increases the long-term survival of transplanted photoreceptors. However, the organization of these cell aggregate transplants was not comparable to the results presented here using intact sheets. The transplantation of intact sheets of fetal neural retina alone to adult RCS rats results in rosette formation or in transplanted photoreceptor degeneration (Aramant and Seiler, unpublished observations, 1998), whereas transplantations of intact retinal sheets to light-damaged rats with intact RPE show development of photoreceptors with inner and outer segments in contact with the host RPE. Using a different approach, another laboratory has recently transplanted "full-thickness" fetal retina to rabbits with normal retina.

The success rate of our experiments was relatively low (4/11) probably for technical reasons, because the surgeon could not see where the tissue was placed in the small rat eye. In addition, because fetal RPE and retina are extraordinarily loosely attached, delicate dissection and transplantation procedures were required. The implantation would be easier in larger animals. However, in contrast to the RCS rat, photoreceptor degeneration proceeds very slowly in the models available in cats, dogs, and pigs. Such animals are expensive and difficult to maintain, and the number of experiments is limited.

Figure 3. Retinal pigment epithelium of retinal cognates. (A, B): Donor age, E18; host age at time of transplantation, 1.8 months; age at time of death, 3.2 months. (C, D) Donor age, E20; host age at time of transplantation, 2.2 months; age at time of death, 3.9 months. (A, C) Enlargement of corotransplanted RPE monolayer in contact with photoreceptor outer segments (OS). Note the clearly demarcated Bruch's membrane (BM). (B, D) Transplanted pigmented RPE sheet (T) on top of presumable host RPE cells (H). There are two Bruch's membranes (BM). (D) The abnormal-appearing host cells have taken up some pigment.
In the present study, the transplanted RPE sheet formed a coherent monolayer that appeared to be continuous with the host RPE at the edges of the transplant area. In contrast, transplantation of injected, dissociated RPE cells often leads to formation of multilayered aggregates that do not show the typical RPE morphology. Tight junctions between RPE cells throughout the retina are critical to the function of the outer blood-retinal barrier. Because in vitro studies showed that the RPE in RCS rats is capable of forming tight junctions, it is possible that the RPE of the host RCS rat formed junctions with the edges of the transplanted RPE sheet. This should be investigated by electron microscopy.

In most areas of the transplant, the transplanted RPE cells were directly opposed to the Bruch's membrane adjacent to the host choroid, and no host RPE could be seen. The host RPE cells may have been scraped off during implantation, or they may have died and have been subsequently removed by macrophages. In some areas, abnormal-looking host RPE cells could be seen underneath a second Bruch's membrane formed by the transplanted RPE sheet. Therefore, it appears that a monolayer could develop in the fetal transplanted RPE sheet without removal of the dysfunctional host RPE.

The normal morphology and organized appearance of the transplant photoreceptors in the present study suggests that transplanted RPE sheets support the development and maintenance of photoreceptor outer segments in the retinal grafts, as in a normal retina, by providing a barrier toward the host choroid and transporting nutrients from the choroid to the photoreceptors. Other studies have shown that the RPE is necessary for normal retinal morphogenesis in vivo and influences the lamination of neural retina in vitro by acting on early Müller glial cells through diffusible factors. The present study confirms that RPE influences retinal organization in vivo and suggests that freshly harvested RPE sheet transplants may...
have a larger rescuing effect on remaining photoreceptors than injected dissociated RPE cells. In some areas, donor RPE was placed on top of host RPE or had folded on itself. However, despite the increased distance from the host choroidal blood supply, transplant photoreceptors in these areas showed development of outer segments. Electron microscopic analysis is needed for closer examination of transplanted RPE and photoreceptor ultrastructure.

Not only is the RPE supportive to photoreceptors, but RPE cells are also affected by photoreceptor activity. For example, the barrier properties and the polarity of embryonic RPE in vitro are dependent on diffusible factors from the neural retina. Therefore, the presence of the cografted neural retina may be important for the development and maintenance of a polarized and functional RPE monolayer.

A healthy RPE is necessary to produce and maintain the integrity of Bruch's membrane. In older patients with eye diseases the diffusion properties of Bruch's membrane are often compromised, resulting in a decreased supply of nutrients to the RPE through Bruch's membrane. Transplanted RPE would have to be able to change the properties of the recipient's Bruch's membrane back to a healthy state.

As a measure of photoreceptor organization and viability, phototransduction molecules are often identified. The level of α-transducin in photoreceptors was shown to decrease rapidly after photoreceptor damage, whereas α-tetragonal and rhodopsin immunoreactivity persisted for 1 to 2 months. In our study, the retinal transplant photoreceptors appeared to have a normal distribution of signal transduction proteins, such as rhodopsin, α-tetragonal, and transducin, and a normal cone interphotoreceptor matrix as shown by PNA staining. The strong staining for α-transducin in organized transplant photoreceptors suggests a normal phototransduction function. The weak α-transducin staining of photoreceptors in rosettes suggested that photoreceptors in rosettes have no or very low light sensitivity. This confirms our previous results that only the photoreceptors supported by RPE and transplanted as fetal intact retinal sheets contain the α-transducin that is necessary to respond to light (see also Ref. 54).

No immune reaction of the recipient was observed around the cografts of RPE with retina when the transplant had been placed correctly without injuring the host Bruch's membrane. This may be because the subretinal space, like the central nervous system, has been considered an immunologically privileged site; that is, it is partially protected from the peripheral immune system. In contrast to photoreceptors, RPE cells can express major histocompatibility complex (MHC) antigens after transplantation and be rejected slowly when they are mismatched in MHC antigens. However, fetal rat RPE cells may not yet express MHC antigens. During transplantation, junctions between the host RPE cells that form the outer blood-retinal barrier were probably broken (in areas where the host RPE had been removed), thus temporarily exposing the transplant to the peripheral immune system in the choroid. At the edges of the transplant, new junctions had to develop between transplant and host RPE cells to restore the outer blood-retinal barrier. The fact that viable transplants were seen at 43 and 51 days after transplantation indicates that no acute rejection had occurred and the outer blood-retinal barrier was most likely intact. However, the possibility of a slow rejection over a longer time frame cannot be excluded. Delayed or slow rejection has been observed in clinical trials of injection of RPE cells to patients with macular degeneration. Future studies should include longer survival times and tissue processing for electron microscopy.

The main conclusions of our study were: first, it is possible to transplant intact sheets of fetal retina and RPE together to the subretinal space; second, both transplanted tissues, retina and RPE, interact to form histologically normal tissue; and third, in the RCS rat, retinal repair requires the cotransplantation of neural retina with RPE to achieve proper photoreceptor transplant development. This procedure offers hope for persons with retinal diseases that affect both RPE and photoreceptors.

The challenge for the future is to achieve functional transplant-host connections and visual improvement.

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References
